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The Glucan, Water Dikinase - A Kinetic Model to Understand the Initial Step in Starch Mobilization in Plant Leaves

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A novel kinetic model for the first enzymatic step committed to the starch degradation pathway in plant leaves is presented and analyzed. It is based on current knowledge and hypotheses about the action and role of glucan, water dikinase (GWD) an essential enzyme for normal starch breakdown. The model is formulated in terms of ordinary differential equations and includes (a) the autoactivation of GWD, (b) the adsorption/desorption to the starch surface, and (c) the interfacial catalysis of the phase transition from crystalline to amorphous sites. It is this transition only, which renders the insoluble starch granule susceptible to hydrolytic attack by exo- and isoamylases, ultimately releasing soluble glucans into the stroma. We identify the efficiency of phase transition as being important for the final state of the system and the adsorption as a potentially feasible regulatory site to control the rate of phase transition and thus starch breakdown.

1 Introduction

Transitory starch in leaves is an important carbon source for sink organs of plants during the night phase. It is composed of amylose and amylopectin with a ratio of approximately 1/5 to 1/9 depending on botanical origin. This composition renders starch rather hydrophobic and insoluble. The granule is degraded during the night by amylolytic enzymes hydrolizing α-1,4-bonds (mainly by β- amylases) and α-1,6-bonds (isoamylases) to release glucans into the stroma.[3] Prior to hydrolytic attack, however, the bonds have to be made accessible. The transition of inaccessible crystalline sites in the interface to accessible amorphous sites is catalyzed by at least one dikinase, the glucan, water dikinase GWD. This enzyme is supposed to unwind glucan double helices at crystalline sites and phosphorylate glucosyl residues to increase the hydrophilicity of starch, thereby preventing spontaneous helix formation after dissociation. This is consistent with an increase of the phosphate content in starch granules at the beginning of the night and a higher activity of amylases on starch in vitro if GWD is provided.[1,2]

2 Motivation

With the model we were able to simulate possible behavior for different conditions and test for the dependence of the phase transition rate on presumably important parameters like the adsorption equilibrium constant. From hereon, we can generate testable quantitative hypotheses to aid and interpret in vitro assays of GWD on starch granules or crystallised maltodextrins, as well as to improve and especially extend our model to include glucan release.

3 Model Formulation

A simple model of the hypothetical initial enzymatic step in starch breakdown is presented. It comprises four reactions describing the catalytic cycle of GWD. The corresponding system of ordinary differential equations is derived.

3.1 Reaction Mechanism

We assume the following simplified reaction scheme:

$$\begin{array}{ll} E + ATP \rightleftharpoons C_1 \rightarrow AMP + C_2 & \text{(autophosphorylation)} \\ C_2 + S_C \rightleftharpoons \Theta & \text{(adsorption/desorption)} \\ \Theta \rightarrow P_i + \alpha S_A + (1-\alpha) S_C + E & \text{(phase transition)} \end{array}$$

At first, ATP is bound reversibly by GWD (E) to build a complex C_1. In the second reaction the β-phosphate of ATP is transferred to a His-residue in the protein yielding the complex C_2 and AMP. After this autoactivation, GWD is adsorbed in the form of C_2 at crystalline sites S_C on the surface. The variable Θ denotes crystalline sites occupied by the enzyme, which can either dissociate again or catalyze the (partial) phase transition to amorphous starch (S_A) in the fourth reaction . This last step lumps together the transfer of one phosphate group to a glucosyl residue, releasing the other phosphate P_i to the bulk phase and desorption of the enzyme. The parameter $\alpha \in (0, 1)$ is introduced in order to cope with the fact that crystalline sites are possibly not transformed into amorphous sites stoichiometrically. Depending on the length of the glucans forming the double helices more than one phosphate may be necessary to get an amorphous site. If for example $\alpha = 0.5$, on average two ATP are consumed for a complete phase transition at a given site.

We assume that the surface of the starch granule is always either crystalline, amorphous or occupied by enzymes. It has to be emphasized that the rates depending on moieties associated with the surface are assumed to be proportional to the corresponding surface area normalized to the maximally available surface area S. This is a usual assumption, which is for example also used in the kinetic derivation of the Langmuir adsorption isotherm.[4]

3.2 Differential Equations

The time evolution of the particle numbers in mole per time follows

$$\frac{d}{dt}\vec{n} = \begin{pmatrix} -1 & 0 & 0 & 0 \\ -1 & 0 & 0 & 1 \\ 1 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & -1 & (1-\alpha) \\ 0 & 0 & 1 & -1 \\ 0 & 0 & 0 & \alpha \\ 0 & 0 & 0 & 1 \end{pmatrix} \vec{v}$$

where $n_1 = [ATP] \cdot V$, $n_2 = [E] \cdot V$, $n_3 = [C_1] \cdot V$, $n_4 = [C_2] \cdot V$, $n_5 = [AMP] \cdot V$, $n_6 = S_C \cdot A_{max}$, $n_7 = \Theta \cdot A_{max}$, $n_8 = S_A \cdot A_{max}$, and $n_9 = [P_i] \cdot V$. The squared

brackets denote concentrations whereas V is the volume of the bulk phase. A_{max} is a parameter characteristic for the adsorbed particle, denoting the maximal GWD adsorption in mol/m^2 if the substrate is in excess.

The rate vector $\vec{v} = (v_1, \ldots, v_4)^T$ was derived assuming mass action kinetics. Simplification of the system description is possible by exploiting two conservation relations, $S \cdot A_{max} = n_6 + n_7 + n_8$, and $N = n_1 + n_3 + n_5$. Eliminating two variables, dividing all equations by V and nondimensionalizing time yield the system

$$
\begin{aligned}
\dot{c}_1 &= \tilde{k}_{-1}(c_1^0 - c_1 - c_4) - \tilde{k}_1 c_1 c_2 \\
\dot{c}_2 &= \tilde{k}_{-1}(c_1^0 - c_1 - c_4) + \tilde{k}_4 \cdot W_{max}(1 - s_c - s_a) - \tilde{k}_1 c_1 c_2 \\
\dot{c}_3 &= \tilde{k}_2(c_1^0 - c_1 - c_4) - W_{max}(K_{ad} \cdot c_3 \cdot s_c - (1 - s_c - s_a)) \\
\dot{c}_4 &= \tilde{k}_2(c_1^0 - c_1 - c_4) \\
\dot{c}_5 &= W_{max} \cdot \tilde{k}_4(1 - s_c - s_a) \\
\dot{s}_c &= (1 - \alpha) \cdot (1 - s_c - s_a) - K_{ad} \cdot c_3 \cdot s_c \\
\dot{s}_a &= \alpha \cdot \tilde{k}_4(1 - s_c - s_a)
\end{aligned}
$$

The variables c_1 to c_5 are the concentrations of ATP, GWD, the complex C_2, AMP, and P_i, respectively. The dimensionless surface ratios are $s_c = S_C/S$ and $s_a = S_A/S$ from which $\theta = \Theta/S$ may be calculated by $\theta = 1 - s_c - s_a$. In the simulations, no AMP is provided initially, therefore c_1^0 denotes the initial ATP concentration. The parameter $W_{max} = \tilde{A}_{max} \cdot \frac{m}{V}$, with m being the mass of starch provided is related to both the space occupied by a molecule of GWD on the starch surface and the shape of the granule. The parameter $K_{ad} = k_3/k_{-3}$ in M^{-1} is the equilibrium adsorption constant of GWD to crystalline sites.

4 Results

4.1 Progress Curves

Figure 1 shows a typical time evolution of the system if enough ATP is provided initially. The crystalline zone is completely transformed into amorphous zone. If the ATP level is too low or the phase transition is inefficient (low α) ATP becomes depleted before transition of the surface is complete (data not shown). This would severely disrupt further breakdown of the starch granule in vivo.

4.2 Control Over the Maximum Rate of Phase Transition

To infer which of the initial reaction steps and associated parameters presumably have a strong impact on the breakdown rate of starch we calculated the influence of some of these parameters on the maximum rate of substrate provision for hydrolysis, namely the maximum phase transition rate $\max v_4$. The results depicted in Figure 2 indicate that regulating the adsorption/desorption constant K_{ad} may be a good means to effectively alter v_4. Also the autophosphorylation step of GWD can be a good regulation site, at least if it is sufficiently slow (data not shown).

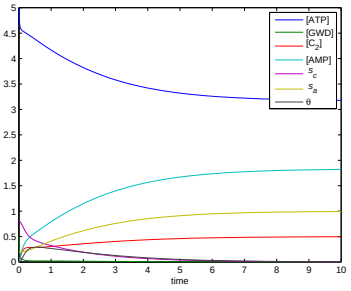

Figure 1. Progress curves of a system with ATP excess. The phase transition is complete and this steady state is stable for many parameter values (data not shown).

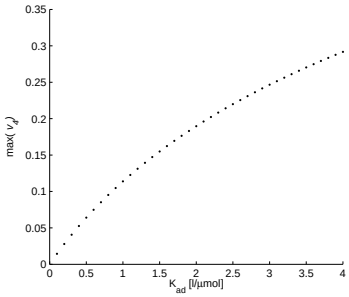

Figure 2. Maximal phase transition rate depending on adsorption equilibrium constant. The positive slope reflects a strong control over the transition rate.

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